TECHNICAL DATA SHEET

THUNDER™ High Performance Phospho STAT6 (Y641) + Total STAT6 TR-FRET Cell Signaling Assay Kit

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BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS KIT-HP-STAT6PT-500 (500 tests)
400 points for phospho-STAT6
and 100 points for total STAT6

Store at -80°C
For research use only.
Not for use in diagnostic procedures.

Visit Product Page

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **phospho-STAT6 (Y641) and total STAT6** protein in cell lysates using a simple, rapid and sensitive immunoassay based on the homogeneous (no-wash) THUNDER™ TR-FRET technology. The kit is compatible with both adherent and suspension cells.

SPECIFICITY

This assay kit contains two specific and selective antibody pairs, one that recognizes **STAT6** phosphorylated at **Tyr641**, and another that recognizes **total** (both phosphorylated and unphosphorylated) **STAT6**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P44226; Entrez-Gene Id: 6778).

Other species should be tested on a case-by-case basis.

TR-FRET ASSAY PRINCIPLE

The **High Performance Phospho-STAT6 (Y641) + Total STAT6** assay kit is a homogeneous time-resolved Förster resonance energy transfer (TR-FRET) sandwich immunoassay (Figure 1). The THUNDER™ Cell Signaling assay workflow consists of 3 steps (Figure 2). Following cell treatment, cells are first lysed with the specific Lysis Buffer 8 provided in the kit. Then **Phospho-STAT6 (Y641) + Total STAT6** in the cell lysates are detected in separate wells with two pairs of fluorophore-labeled antibodies in a simple "add-incubate-measure" format (single-step reagent addition; no wash steps). For detection of the phosphorylated protein, one antibody is labeled with a donor fluorophore (Europium chelate; Eu-Ab1) and the second with a far-red acceptor fluorophore (FR-Ab2). The same approach is used for the second antibody pair detecting the total protein (Eu-Ab3 and FR-Ab4). The binding of the two matched labeled antibodies to distinct epitopes on the target protein (either **phospho-STAT6** or **total STAT6**) takes place in solution and brings the two dyes into close proximity. Excitation of the donor Europium chelate molecules with a flash lamp (320 or 340 nm) or a laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which in turn emit a TR-FRET signal at 665 nm. Residual energy from the Eu chelate generates light at 615 nm. The signal at 665 nm is proportional to the concentration of **Phospho-STAT6 (Y641)** and **Total STAT6** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm rationm/615 nm ratio.

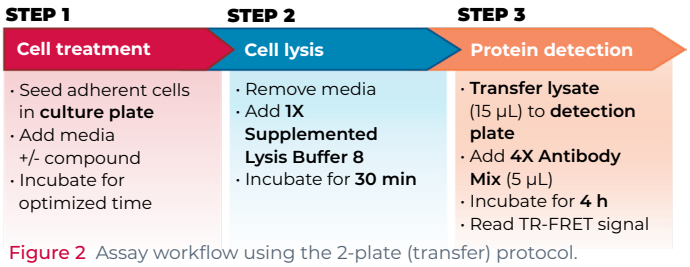


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

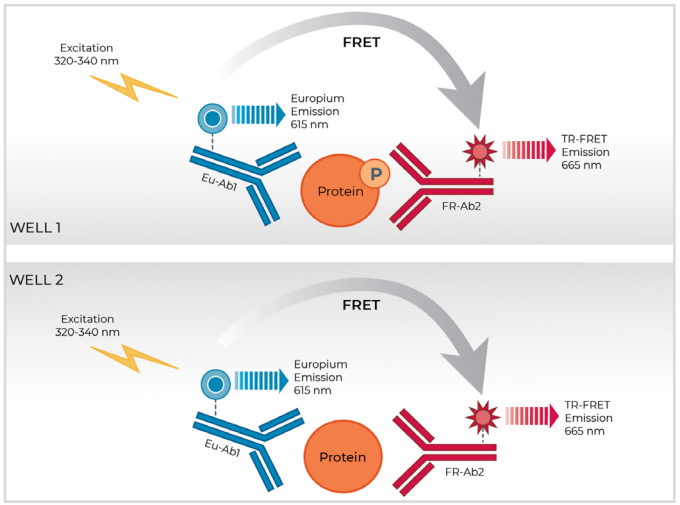


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

KIT COMPONENTS	500 points*
Eu-labeled HP-phospho-STAT6 (Y641) antibody (Eu-Ab1)	20 µL
Acceptor-labeled HP-phospho-STAT6 (Y641) antibody (FR-Ab2)	80 µL
Eu-labeled HP-total-STAT6 antibody (Eu-Ab3)	5 µL
Acceptor-labeled HP-total-STAT6 antibody (FR-Ab4)	20 µL
Lysis Buffer 8 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	250 µL

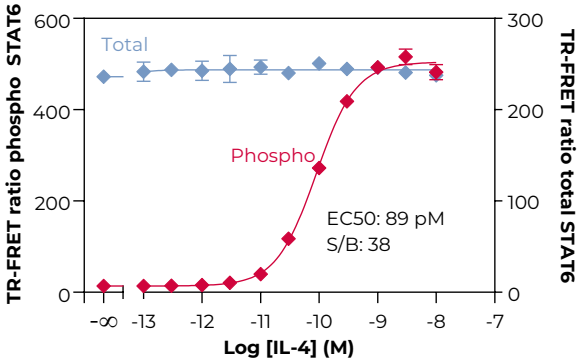
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA IN HELA CELLS

This assay kit has been validated for the relative quantification of phospho STAT6 (Y641) and total STAT6 in HeLa cell lysates using the 2-plate assay protocol.

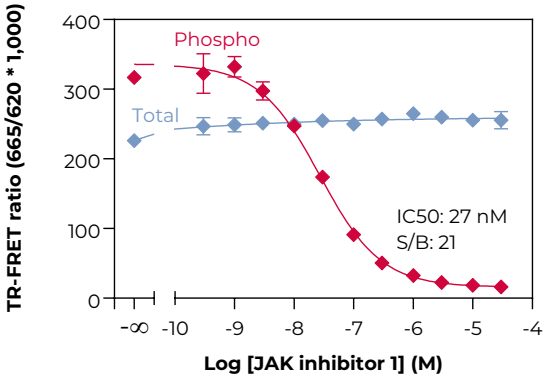
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 8** (50 µL) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X.
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were transferred to a 384-well assay plate followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho STAT6 (Y641) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total STAT6.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (PHERASTAR® FSX; laser excitation).

STIMULATION OF PHOSPHO-STAT6 (Y641) IN HELA CELLS



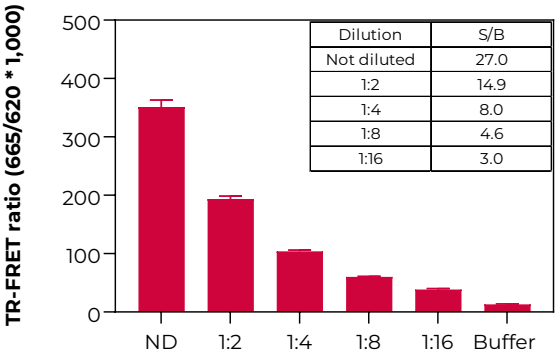
HeLa cells (100,000 cells/well; in triplicate) were incubated with serial dilutions of IL-4 for 30 min at RT. Data show that treatment of HeLa cells with IL-4 stimulates phosphorylation of STAT6 at Y641, but does not affect the levels of total STAT6.

INHIBITION OF PHOSPHO-STAT6 (Y641) IN HELA CELLS



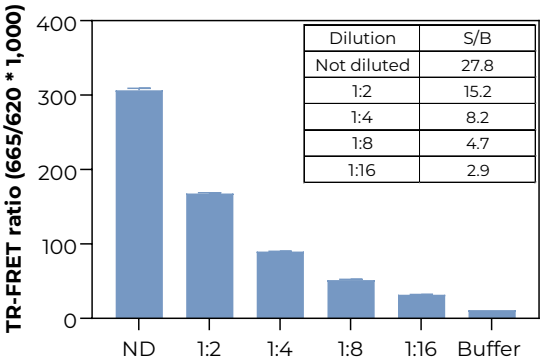
HeLa cells (100,000 cells/well; in triplicate) were incubated with serial dilutions of JAK Innibitor 1 for 30 min at RT. Cells were then stimulated with 0.2 nM of IL-4 for 30 min at RT. Data show that treatment of HeLa cells with JAK Innibitor 1 inhibits phosphorylation of STAT6 at Y641 by IL-4, but does not affect the levels of total STAT6.

HELA CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-STAT6 (Y641)



Dilution	S/B
Not diluted	27.0
1:2	14.9
1:4	8.0
1:8	4.6
1:16	3.0

HELA CONTROL LYSATE TITRATION (QC TEST) TOTAL STAT6



Dilution	S/B
Not diluted	27.8
1:2	15.2
1:4	8.2
1:8	4.7
1:16	2.9

Quality Control: the High Performance Phospho-STAT6 (Y641) and Total STAT6 assay kit is routinely tested against IL-4 treated HeLa lysates. HeLa cells were cultured in a T175 flask to 100% confluency and stimulated with 3 nM of IL-4 for 20 min at RT. Following cell lysis using 5 mL of 1X Lysis Buffer 8, lysates were serially diluted with 1X Lysis Buffer 8 and tested in triplicate. Data show a linear relationship between lysate dilutions and TR-FRET ratio values.

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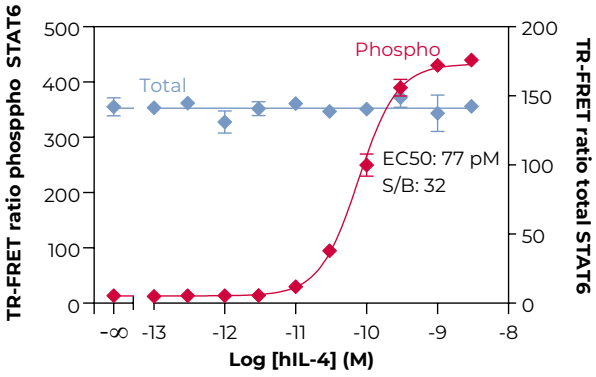
VALIDATION DATA IN THP-1 CELLS

This assay kit has also been validated for the relative quantification of phospho STAT6 (Y641) and total STAT6 in THP-1 cell lysates using the 2-plate assay protocol.

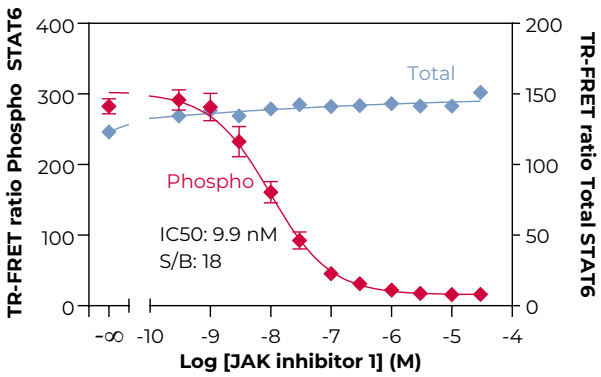
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifguged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, the cells were lysed with the 5X **Lysis Buffer 8** supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 5X.
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were transferred to a 384-well assay plate followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of total STAT6.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (PHERASTAR® FSX; laser excitation).

STIMULATION OF PHOSPHO-STAT6 (Y641) IN THP-1 CELLS



THP-1 cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of IL-4 for 30 min at RT. Data show that treatment of TPH-1 cells with IL-4 stimulates phosphorylation of STAT6 at Y641, but does not affect the levels of total STAT6.

INHIBITION OF PHOSPHO-SAT6 (Y641) IN THP-1 CELLS



THP-1 cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of JAK Innibitor 1 for 30 min at RT. Cells were then stimulated with 0.25 nM of IL-4 for 30 min at RT. Data show that treatment of HeLa cells with JAK Innibitor 1 inhibits phosphorylation of STAT6 at Y641 by IL-4, but does not affect the levels of total STAT6.

FOR MORE INFORMATION ON DEVELOPING AND OPTIMIZING TR-FRET CELL SIGNALING ASSAYS, CONSULT THE USER MANUAL.

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